Epidermal Langerhans Cell Depletion After Artificial Ultraviolet B Irradiation of Human Skin *In Vivo*: Apoptosis *Versus* Migration

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Ultraviolet B radiation can suppress cellular immunity. One of the mechanisms related to this immunosuppression is the disappearance of Langerhans cells from the epidermis. The aim of this study was to establish the mechanism of ultraviolet B-induced Langerhans cell disappearance in healthy individuals. The two most likely mechanisms for Langerhans cell disappearance are apoptosis and migration. Apoptosis was assessed *in vivo* by exposing buttock skin of 10 healthy volunteers to six minimal erythema doses of ultraviolet B. Only very few apoptotic Langerhans cells could be observed in sections from the ultraviolet B-exposed skin. Migration of Langerhans cells cannot be established in skin sections and suction blisters were therefore raised in an attempt to trap migrating Langerhans cells in the sub-basal membrane blister fluid. Blisters were raised on the flexor side of the lower arm of 30 healthy volunteers at several time points after exposure of the skin to six minimal erythema doses of ultraviolet B. Blister fluid was collected and blister roofs were removed to check for Langerhans cell disappearance. Langerhans cells were detected in the blister fluid of the ultraviolet B-exposed skin and not of the unexposed skin. The number of Langerhans cells in the blister fluid peaked at about 18 h after ultraviolet exposure, which coincided with the largest depletion of Langerhans cells in the blister roof. A fraction (20–30%) of the Langerhans cells in the blister fluid stained positive for DNA damage (cyclobutyl pyrimidine dimers), showing that they originated from the epidermis. Ultraviolet B-induced Langerhans cell disappearance appears to be mainly attributable to migration. ***Key words: caspase/pyrimidine dimers/suction blister/ultraviolet immunosuppression. J Invest Dermatol 118:812–817, 2002***

Ultraviolet (UV) radiation, especially UVB radiation (280–315 nm) has several effects on the skin immune system. There are several primary photochemical reactions that could trigger these events. The most studied are: isomerization of urocanic acid and DNA damage (cyclobutyl pyrimidine dimers and 6–4 photoproducts) in epidermal cells. Subsequently, the production of cytokines is induced, such as interleukin-1β, tumor necrosis factor-α, interleukin-10, and prostaglandin E_2. Cellular functions are altered, e.g., natural killer cell activity is inhibited, antigen presentation is changed, and the microenvironment becomes favorable to the development of T-helper 2-like immune responses (for review Duthie *et al*, 1999). (Over)exposure of the skin to UVB leads to an influx of interleukin-10 producing macrophagic cells (Kang *et al*, 1994, 1998) and a depletion of Langerhans cells. These effects are associated with suppression of cellular immune reactions.

A disturbance in these immunosuppressive mechanisms may lead to the development of a UV-induced "sun allergy" (polymorphous light eruption). Patients with polymorphous light eruption are sensitive to UVB (as clinically tested by challenges with a Philips TL12 lamp), UVA, and sometimes even to visible light. These patients develop papules, vesicles, or eczema on sun-exposed areas of the skin (Epstein, 1997; Grabczyska and Hawk, 1997; Salomon *et al*, 1997; Boonstra *et al*, 2000). Polymorphous light eruption is suggested to be a delayed-type hypersensitivity reaction (Epstein, 1997; Norris *et al*, 1988; Verheyen *et al*, 1995). In earlier experiments we found that Langerhans cells in the skin of polymorphous light eruption patients persisted after overexposure to UVB in contrast to what was observed in healthy volunteers (Kölgen *et al*, 1999). We hypothesize that this persistence of Langerhans cells in the epidermis may contribute to the pathogenesis of the disease. To understand this defect in polymorphous light eruption patients, the main mechanism that is responsible for the UV-induced Langerhans cell disappearance in healthy individuals needs to be established.

The paramount mechanism underlying Langerhans cell depletion, especially in humans, is still unknown. Some authors suggest that a deficiency of growth factors for Langerhans cells or a downregulation of growth factor receptors is responsible for Langerhans cell depletion (Takashima, 1995). The best studied and two most likely mechanisms for epidermal Langerhans cell depletion are apoptosis and/or migration.

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Abbreviations: AP, alkaline phosphatase; AEC, 3-amino-ethyl-carbazole.

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In vitro experiments revealed that irradiated human Langerhans cells undergo apoptosis (Rattis *et al*, 1998). Experiments with mice showed that apoptosis of Langerhans cells is induced by UVB radiation through, among others, the production of reactive oxygen species (Takashima, 1995). Whether this also occurs in humans *in vivo* has still not been demonstrated (Meunier, 1999).

We know from animal models that Langerhans cells migrate to the lymph nodes after exposure of the skin to UVB (Sontag *et al*, 1995; Dandie *et al*, 1998). UV-induced migration of Langerhans cells in humans has been described by Yawalkar *et al*, 1998). These last experiments (performed by Yawalkar), however, involve lymph drainage, and are very encumbering and difficult to perform; moreover, they were not approved of by the ethical committee of our institute. We therefore had to resort to a novel technique.

To investigate the relative importance of apoptosis *vs* migration in UV-induced Langerhans cell depletion *in vivo* we studied a group of healthy volunteers. A small area of the buttock skin or flexor side of the arm was overexposed to UVB. Biopsies were taken from buttock skin at successive time points after exposure. Furthermore, we developed a novel method to detect migrating Langerhans cells *in vivo* by catching them in blister fluid: suction blisters were raised on (un)exposed areas of the skin of the flexor lower arm, and blister roofs and blister fluid were collected at several time points after UVB exposure. Immunohistochemical stainings for CD1a (Langerhans cells), cyclobutyl pyrimidine dimers (DNA damage), and for active caspase 3 and terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL) (both markers for apoptosis) were performed on frozen skin sections, blister roofs, and cytospins of blister fluid.

MATERIALS AND METHODS

Subjects Thirty healthy individuals (nine males and 21 females, aged between 19 and 55 y) were studied for Langerhans cell migration (see below), whereas 10 healthy individuals (three males and seven females, aged between 22 and 31 y) were studied for apoptosis. Volunteers whose buttock skin or skin of the lower arm was exposed to sunlight less than 2 mo ago were excluded. Informed consent was obtained from all volunteers. This study was approved by the Medical Ethical Committee of the University Medical Center, Utrecht, the Netherlands.

Phototesting procedure The UV dose required to cause a just perceptible redness, the minimal erythema dose (MED), was determined on the skin area to be experimented on, i.e., the buttock skin or flexor side of the lower arm. For MED determination a test device with nine windows (3 × 10 mm each) was used. Through these windows the underlying skin was exposed to a Philips TL12 lamp for different periods of time (a geometrical series, e.g., from 12.4 to 200 s). The TL12 lamp emitted 58% of the UV output in UVB (280–315 nm) and 5% of the UV output below 290 nm. Erythemally weighted, 98% stemmed from the UVB band (18% below 290 nm). The erythema was assessed 24 h later. The MED for the buttock skin ranged from 212 to 750 J per m^2 UVB (median of 410 J per m^2, 95% CI 180–940 J per m^2). The MED for the flexor side of the arm was four to five times higher and ranged from 352 to 4840 J per m^2 UVB (median of 1650 J per m^2, 95% CI 470–5820 J per m^2). After determination of the MED the test areas of skin on the buttock or lower arm were exposed to 6 MED UV (Philips TL12). According to Barr *et al* (1999) this dose is about equal to 4 MED for an erythema defined by clear borders, as used by Cooper *et al* (1992) to suppress contact hypersensitivity in humans.

Biopsies Three millimeter punch biopsies were taken from the irradiated buttock skin 24 h and 48 h after exposure to 6 MED UV, together with one control biopsy from the unexposed buttock skin. Biopsies were snap frozen in liquid nitrogen, embedded in OCT compound (Tissue-Tek, Sakura, Zoeterwoude, the Netherlands) and stored at –30°C until further processing.

Suction blisters Suction blisters were raised (van der Leun *et al*, 1974; Black *et al*, 1977) for operational convenience on the irradiated skin of the flexor side of the lower arm at several time points after exposure to 6 MED UV (18 h, 24 h, 30 h, 42 h, 48 h) and on an unexposed side (two blisters per volunteer). Blisters were raised over a period of about 2 h, using airtight cups with circular ports exposing skin areas of 10 mm in diameter to an atmospheric pressure of –200 mmHg. Suction blister formation was further stimulated by gently warming the skin via an electric heating coil in each suction cup. Skin temperature was measured with a copper-constantan thermocouple touching the skin, and did not exceed 40°C. Blisters occurred at a dermal–epidermal junction. Blister fluid was collected using a microneedle with a diameter of 0.36 mm. Blister roofs were removed with a sterilized skin surgery set. Each volunteer was sampled at only one time point after irradiation. The yield of blister fluid varied among individuals, but slightly more blister fluid was consistently recovered from UV-irradiated blisters when compared with controls, as found earlier by Barr *et al* (1999). Cytospin preparations were made of the blister fluid by centrifugation for 10 min at 55 × *g* on to 3-amino-propyltriethoxysilane-coated glass slides. The slides were stored at –30°C until further processing. The removed blister roofs were used immediately for immunohistochemistry.

Antibodies As primary antibodies, monoclonal antibodies CD1a (DAKO A/S, Glostrup, Denmark, diluted 1:60), fluorescein isothiocyanate (FITC)-conjugated CD1a (DAKO A/S, diluted 1:60) and biotinylated H3 antibody (directed against cyclobutyl pyrimidine dimers, a kind gift from Dr L. Roza; T.N.O., Zeist, the Netherlands; diluted 1:20), and the polyclonal antibody rabbit anti-active caspase 3 (Becton Dickinson, San Diego, CA, diluted 1:100) were used. Biotinylated horse anti-mouse immunoglobulin (Vector, Burlingame, CA; diluted 1:800) and biotinylated goat anti-rabbit antibody (Vector; diluted 1:300) were used as secondary antibodies. Alkaline phosphatase (AP)-labeled F(ab) fragments of sheep anti-FITC (Boehringer Mannheim GmbH, Mannheim, Germany; diluted 1:400), AP-labeled avidin–biotin complex (DAKO A/S; diluted 1:50) horseradish peroxidase (HRP)-conjugated avidin–biotin complex (DAKO A/S; diluted 1:50) were used as detecting reagents.

Immunohistochemistry

Single staining of CD1a and anti-active caspase 3 Blister roofs and frozen skin sections (6 μm) on 3-amino-propyltriethoxysilane-coated glass slides were fixed for 10 min at room temperature in dry acetone containing 100 μl 30% H_2O_2 per 100 ml. The slides were incubated for 20 min with a blocking reagent [phosphate-buffered saline (PBS) containing 10% normal human serum/10% normal horse serum or 10% normal goat serum (depending on the secondary antibody species)] to prevent

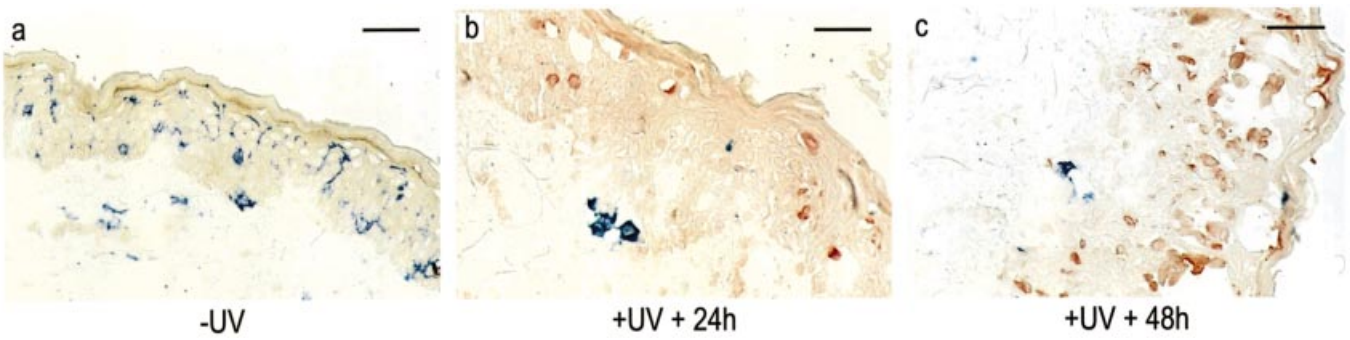

Figure 1. Double staining of CD1a (*blue*) and active-caspase 3 (*red*) of buttock skin, unexposed (– UV), 24 h (+ UV + 24 h) and 48 h after UVB exposure (+ UV + 48 h). *Scale bar*: 50 μm.

aspecific binding. The skin sections were subsequently incubated with the primary antibody (CD1a or rabbit-anti active caspase 3) for 1 h and with a secondary antibody (biotinylated horse anti-mouse immunoglobulin or biotinylated goat anti-rabbit immunoglobulin, respectively) for 1 h. Subsequently, the blister roofs and skin sections were incubated for 30 min with HRP-labeled avidin–biotin complex (CD1a) or AP-labeled avidin–biotin complex (active caspase 3). Peroxidase activity was visualized by incubation in 0.1 M acetate buffer (pH = 5) containing 20 mg 3-amino-9-ethyl-carbazole (AEC; Sigma, St Louis, MO) and 100 µl H_2O_2 per 100 ml, resulting in a red staining. Rabbit anti-active caspase 3 binding was visualized by incubating the sections in Tris–HCl (pH = 8.5) containing 25 mg levamisole, 250 µl New Fuchsin, 250 µl 0.6 M sodium nitrate solution and 50 mg naphthol AS-BI phosphate, resulting in a pink staining.

Double staining of CD1a-FITC and H3-biotin CD1a-FITC/H3 double staining was performed as described previously (Sontag *et al*, 1995) without modifications. In short, blister roofs, cytospins of blister fluid, and frozen skin sections were fixed, preincubated with blocking reagent (PBS containing 10% normal human serum) and incubated with the primary antibody CD1a-FITC, as described above. After incubation for 1 h with AP-labeled sheep anti-FITC F(ab) the AP activity was visualized by incubation in a BCIP/NBT solution of 5-bromo-4-chloro-3-indolyl phosphate/4-nitro blue tetrazolium chloride in 0.1 M Tris–HCl (pH = 9.5), 0.1 M NaCl, 0.05 M $MgCl_2$, and 2.5 mg levamisole per 10 ml. The bluish-black reaction product is water and ethanol insoluble and withstands the subsequent harsh treatment. The preparations were fixed in 25% acetic acid (vol/vol) containing 100 µl 30% H_2O_2 per 100 ml. After washing two times for 10 min in PBS the slides were incubated for exactly 2 min in 0.07 M NaOH in 70% ethanol. After washing with PBS the preparations were incubated for 20 min with PBS containing 10% normal human serum and 10% normal mouse serum, followed by a 1 h incubation with H3-biotin.The preparations were incubated with HRP-labeled avidin–biotin complex for 30 min and developed in an AEC substrate solution, as described above.

Double staining of CD1a-FITC and anti-active caspase 3 CD1a-FITC/active caspase 3 double staining was performed on blister roofs and frozen skin sections. Fixation and preincubation of the skin sections was performed as described above. The sections were then incubated simultaneously with CD1a-FITC and anti-active caspase 3 antibody for 1 h. The preparations were subsequently incubated simultaneously with biotinylated goat-anti-rabbit and AP-labeled sheep anti-FITC for 1 h, followed by incubation for 30 min with HRP-labeled avidin–biotin complex. AP activity was visualized by incubating the sections first in a Tris–HCl buffer (pH = 8.5), containing 25 mg Fast Blue BB Salt, 12.5 mg naphthol AS-MX phosphate, and 35 mg levamisole per 100 ml (all purchased from Sigma), resulting in a blue staining. For visualization of the peroxidase activity the sections were then incubated with an AEC solution, as described above. Negative staining controls were used in single and double staining experiments in which the first antibody was omitted or replaced by an irrelevant antibody of the same isotype. All antibodies were diluted in the suitable blocking reagent except for the AP- and HRP-labeled antibodies, which were diluted in PBS/0.05% Tween 20. All antibody incubations were performed in a humidified chamber at room temperature. After each antibody incubation the slides were washed three times with PBS containing 0.05% Tween 20.

TUNEL To detect DNA breaks *in situ*, TUNEL staining was performed using a commercially available detection kit (Boehringer Mannheim GmbH). Frozen skin sections (6 µm) on 3-amino-propyltriethoxysilane-coated glass slides were stained according to manufacturer's instruction. Incorporation of FITC labeled nucleotides was checked using a Zeiss microscope equipped for epifluorescence. Converter-AP (anti-FITC antibody supplied in the kit) was then added and antibody binding was visualized by incubation in a Fast Blue BB Salt substrate solution, as described above. To detect whether the apoptotic cells were Langerhans cells the TUNEL assay was combined with a CD1a staining. CD1a antibody was incubated simultaneously with the TdT reaction mixture. The sections were then incubated simultaneously with biotinylated horse anti-mouse immunoglobulin and converter-AP followed by incubation with HRP-labeled avidin–biotin complex. Antibody binding was visualized by incubation with Fast Blue BB Salt substrate solution (TUNEL) and AEC solution (CD1a), as described above.

Statistical analysis After logarithmic transformation (to correct for the right skewed distribution) a Student's t test was performed to ascertain the significance of the observed differences (significance level $p < 0.05$). The numbers of CD1a single positive and CD1a/H3 double positive cells in the blister fluid after UV exposure were compared with the numbers of unexposed, control skin.

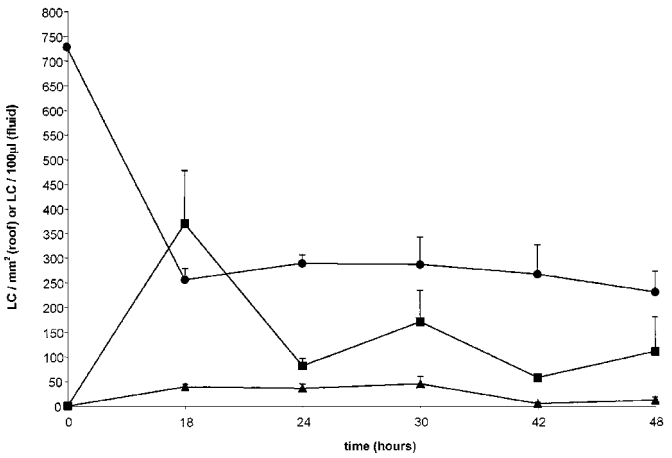

Figure 2. Number of CD1a$^+$ Langerhans cells (mean ± SEM) in blister roofs (bullet) and blister fluid (CD1a$^+$ cells, ■ and CD1a$^+$/H3$^+$ cells, ▲, of arm skin, unexposed (0 h) and UVB-exposed (18 h, 24 h, 30 h, 42 h, 48 h). Average n per time-point = 5.

RESULTS

Exposure to 6 MED causes apoptosis in the epidermis, but not Langerhans cells Caspase 3 is an effector caspase that is cleaved during apoptosis in a wide variety of cells and commits a cell to apoptosis (for review see Harvey and Kumar, 1998; Thornberry and Lazebnik, 1998).

Active caspase 3-positive cells are present in the epidermis 24 h and 48 h after UVB irradiation and are abundantly present in blister roofs within 18 h after 6 MED UVB irradiation. The number of caspase 3-positive cells 24 h after UVB exposure varies greatly among volunteers. Active caspase 3-positive cells are found scattered throughout the epidermis 24 h after irradiation, whereas after 48 h the cells are mainly located in the upper part of the epidermis just below the stratum corneum (**Fig 1**). These apoptotic cells are mainly found in conjunction with epidermal defects. Active caspase 3-positive cells were not found in unirradiated skin. Similar results were obtained with the TUNEL staining, but the TUNEL assay had a higher background staining (data not shown).

To test whether the caspase 3-positive cells found in the UVB-irradiated skin were Langerhans cells, a double staining was performed with the dendritic cell marker CD1a. No double positive cells could be detected in the UVB-irradiated skin (**Fig 1**). The Langerhans cells tended to be low in the epidermis or in the dermis, whereas the apoptotic cells were higher up in the epidermis. A few (< 1% of the total number of Langerhans cells from unirradiated skin and < 3% of the total number of Langerhans cells from UVB-exposed skin) caspase 3-positive or TUNEL-positive Langerhans cells could occasionally be observed in the blister roofs of the UVB-irradiated skin.

CD1a$^+$ Langerhans cells can be detected in the blister fluid of the UVB-exposed skin, but not of the unexposed skin To test whether UVB-induced Langerhans cell depletion was caused by migration, suction blisters were raised on UVB-irradiated skin and unirradiated skin. Blister roofs and blister fluid were sampled at several time points after irradiation. CD1a staining of blister roofs showed a significant decline ($p < 0.0001$) in the number of Langerhans cells after UVB irradiation (median of 259 cells per mm^2, 95% CI 146–462 cells per mm^2) when compared with the unirradiated control (median of 720 cells per mm^2, 95% CI 520–996 cells per mm^2) (**Fig 2**). The largest decrease appeared

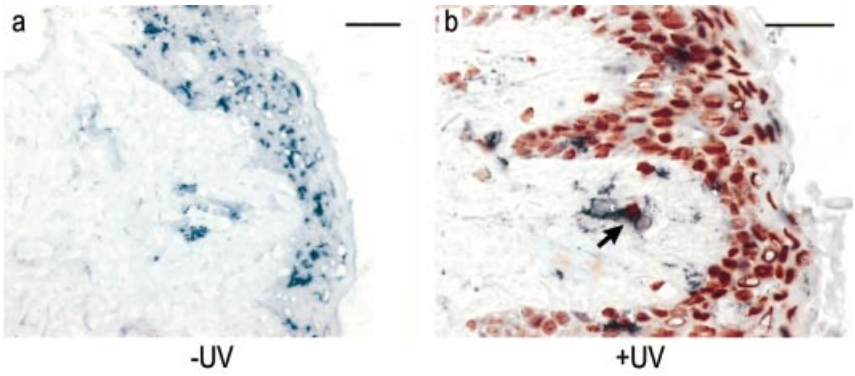

Figure 3. Double staining of CD1a (*blue*) and H3 (*red*) of buttock skin, unexposed (– UV) and 24 h after UVB exposure (+ UV) with an example of a doubled stained cell, migrating out of the epidermis (*black arrow*). *Scale bar*: 50 μm.

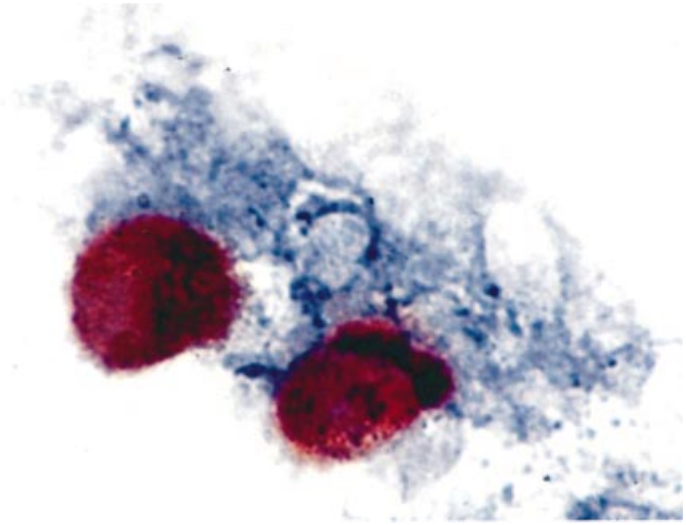

Figure 4. Double staining of CD1a (*blue*) and H3 (*red*) in blister fluid of the UVB-exposed skin.

to occur over the first 18 h period (60%), whereas between 18 h and 48 h there was a further decrease of only 10%.

Blister fluid was sampled to collect migrating Langerhans cells. Very few other cells were detected in the fluid (except for a minor admixture of about 50 cells in three of 30 cases in which blisters developed more quickly within about 1.5 h). CD1a staining performed on cytospin preparations of blister fluid showed a significant difference ($p < 0.0001$) between UVB-irradiated and unirradiated skin. $CD1a^+$ Langerhans cells could be detected in the blister fluid of the UVB-exposed skin, but not in the blister fluid of the unirradiated skin. The number of $CD1a^+$ cells varied strongly among the healthy individuals (median of 115 cells per 100 μl sample, 95% CI 17–796 cells per 100 μl sample) (**Fig 2**). The blister fluid from the earliest time point (18 h) showed the largest number of Langerhans cells (median of 268 cells per 100 μl sample, 95% CI 44–1623 cells per 100 μl sample); however, the number of Langerhans cells that were found in the blister fluid of an individual did not correlate with the number of Langerhans cells that disappeared from the blister roofs of the UVB-exposed skin (rho = – 0.355, p = 0.089, Spearman rank order correlation test). The individual UVB doses (in J per m^2) showed a negative correlation with the numbers of Langerhans cells in the blister fluid (rho = – 0.421, p = 0.04, Spearman rank order correlation test), whereas the UVB doses showed a positive correlation with the decrease of Langerhans cells in the blister roofs (rho = 0.565, p = 0.001, Spearman rank order correlation test). The maximum number of Langerhans cells in the blister fluid at 18 h coincided with the largest decrease in Langerhans cells in the blister roof.

Cyclobutyl thymidine dimers are detectable 18 h after 6 MED UVB in epidermal cells but only in a fraction of the migrating Langerhans cells Skin biopsies, blister roofs, and blister fluid were stained with H3 to detect UV-induced DNA damage, containing thymidine dimers. After a single dose of 6 MED UVB most cells in blister roofs and skin biopsies are $H3^+$. Blister roofs or skin biopsies from unexposed skin did not contain $H3^+$ cells (**Fig 3**). H3 staining was performed on cytospin preparations of blister fluid to confirm that the $CD1a^+$ cells originated from the UV-exposed epidermis. CD1a/H3 double positive cells could be observed in the blister fluid at different time points (18 h–48 h) after UV irradiation (**Fig 4**). But only a minority of the $CD1a^+$ cells was $H3^+$ (mean of 27%). The percentage CD1a/H3 double positive cells of the total amount of $CD1a^+$ cells in the blister fluid was least at 42 h (15.3%) and greatest at 24 h (38.8%) (**Fig 2**). Because of layering of cells and excessive H3 staining it was difficult to interpret the CD1a/H3 staining in skin sections and whole blister roofs. Therefore, single cell suspensions of blister roofs were double stained for CD1a and H3 to check whether Langerhans cells in the UV-exposed skin also contained UV-damaged DNA (**Fig 3**). The majority of the $CD1a^+$ cells in single cell suspensions of blister roofs were $H3^+$.

DISCUSSION

It is well known that UVB can induce immunosuppression in the skin. One of the important processes associated with immunosuppression is the disappearance of the primary antigen-presenting cells of the skin, the Langerhans cells. Here we investigated whether apoptosis or migration dominated this prominent effect on the epidermal antigen-presenting cells. The disruption of Langerhans cell function can be fundamental to UV-induced immunosuppression either by incorrect, inappropriate, or inefficient antigen presentation (Duthie *et al*, 1999). Alternatively, depletion of Langerhans cells could lead to modified antigen presentation by another cell type, e.g., infiltrating monocytoid cells (Meunier *et al*, 1995).

In this study we first focused on the role of apoptosis in UVB-induced Langerhans cell depletion, as the occurrence of this phenomenon in humans *in vivo* still has not convincingly been demonstrated (Meunier, 1999). We show that only a few epidermal $CD1a^+$ Langerhans cells in skin biopsies or blister roofs undergo apoptosis after UVB exposure, as detected by active-caspase 3 or TUNEL. These results are in line with experiments of Okamoto *et al* (1999), who showed in mice that after UV radiation only 1.5 cell per 900 Langerhans cells per mm^2 were apoptotic, which was far too few to contribute substantially to the observed Langerhans cell depletion. Hollis and Scheibner (1988) found that Langerhans cells in UV-irradiated skin of Aboriginals were apoptotic. The evidence is, however, circumstantial as the number of apoptotic Langerhans cells was very low and the time frame used was not optimal (2 h or 5 d). *In vitro* experiments demonstrated that Langerhans cells did go into apoptosis after UVB irradiation and 2 d of culture (Rattis *et al*, 1998). Tang and Udey (1992a) found that death of cultured murine

Langerhans cells is augmented by UVB irradiation. A population of HLA-DR$^+$ cells migrating from cultured, *ex vivo* irradiated skin explants are shown to be apoptotic (Nakagawa *et al*, 1999). Mommaas *et al* (1993) showed by electron microscopy that some human Langerhans cells become necrotic when irradiated with UVB *in vitro*; however, necrotic cells also become TUNEL$^+$, but were not detected as Langerhans cells in our study. Furthermore, lack of swollen, disintegrated Langerhans cells (detected by CD1a, Lag, or HLA-DR; data not shown) indicates that necrosis is negligible for UV-induced Langerhans cell depletion.

A possible explanation for the difference in UV-induced death of Langerhans cells between the *in vitro* or *ex vivo* and the *in vivo* situation could be the difference in environment. In human skin, which consists of several layers of epidermal cells, Langerhans cells might be protected by the surrounding cells. This might be due to protective shielding, but also by cell–cell contacts, cell surface molecules, and cytokines that are lacking or different *in vitro* (Tang and Udey, 1992b; Laihia and Jansen, 2000). One could argue that the CD1a marker may be lost during apoptosis and that this is the reason why we could hardly detect any apoptotic CD1a$^+$ cells. Nakagawa *et al* (1999) showed in their *in vitro* experiments, however, that annexin V$^+$ apoptotic cells from UVB-irradiated skin exhibited a high expression of CD1a, which would imply that apoptotic Langerhans cells retain their CD1a surface marker. Taken together, our data show that apoptosis occurs abundantly in human epidermis after overexposure to UVB; however, Langerhans cells apparently do not go into apoptosis and this mechanism can therefore not explain the UVB-induced depletion of Langerhans cells in the epidermis.

The next step in unraveling the mechanism of UVB-induced Langerhans cell depletion was investigating Langerhans cell migration. Pilot experiments had shown that it is feasible to catch migrating Langerhans cells *in vivo* in the fluid of suction blisters. Using this method we were indeed able to detect Langerhans cells with pyrimidine dimers reproducibly in the blister fluid of the UVB-exposed skin, and not in the blister fluid of the unexposed skin. Blocking the emission of the Philips TL12 lamps below 290 nm (using a WG305/3 and a WG295/1 filter simultaneously) to simulate a solar-simulating irradiation source resulted in an equal number of Langerhans cells in the in blister roofs and blister fluid of the UV-exposed skin as compared with the unfiltered TL12 lamps (tested on two people, data not shown). The decrease in Langerhans cell number in the blister roof after UVB radiation did not correlate with the number of Langerhans cells found in the blister fluid of the UVB-exposed skin, although the largest decrease in the blister roof coincided with the largest increase in Langerhans cell number in the blister fluid. The correlation is, however, likely to be affected by factors such as variations between individuals in time over which blisters were raised, differences in adherence of Langerhans cells to the dermal blister floor, amount of blister fluid, and blister cross-section.

The presence of cyclobutyl pyrimidine dimers in the CD1a$^+$ cells in the blister fluid confirmed that the cells originated from the UVB-exposed epidermis. Overall 27% of Langerhans cells in the blister fluid, but most of the epidermal cells (mainly keratinocytes), had detectable levels of pyrimidine dimers. One could think of several explanations for this low percentage of Langerhans cells with detectable pyrimidine dimers. First of all, one could speculate that the CD1a single positive cells do not originate from the epidermis but are new dendritic cells coming from the blood stream or hair follicles (Gilliam *et al*, 1998), or entering the blister fluid as a result of the suction blister technique. It is, however, unlikely that the undamaged Langerhans cells come from the dermis as their dominant occurrence in blister fluid does not coincide with a repopulation of the epidermis (Kölgen *et al*, 1999). Furthermore, it does not appear to be an artifact of the method used, no influx of Langerhans cells could be observed in the blister fluid of the unexposed skin. Moreover, cytokeratin 14 staining on basal keratinocytes in the blister roof of the UVB-irradiated skin showed that the integrity of the blister roof was not affected by the method used nor by UVB irradiation (data not shown). Hence, Langerhans cells are not likely to be released from the epidermis through defects caused by the blister formation. Alternatively, it could be that the Langerhans cells had a low initial damage level (Vink *et al*, 1994) or a rapid DNA repair. The undamaged Langerhans cells may show selective responsiveness to migratory signals, such as tumor necrosis factor-α. By staining single cell suspensions of blister roofs (18 h time point) it was indeed possible to see a clear enrichment of DNA-damaged Langerhans cells (approximately 80–90%) in blister roofs compared with blister fluid. Of note is the difference in percentage of double positive cells between 18 h (20.1% of Langerhans cells) and 24 h (38.8% of Langerhans cells). These percentages are mainly influenced by the number of CD1a single positive cells because the absolute number of damaged Langerhans cells in the blister fluid is almost the same between 18 h and 30 h. This also suggests that undamaged Langerhans cells are quicker to migrate.

In this study, it has been shown that raising suction blisters is an easy-to-use method to catch migrating Langerhans cells in human beings *in vivo*. These data show that UVB-induced Langerhans cell depletion in human skin is mainly caused by migration and not by apoptosis.

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